

Serial No. 10/612,298

Attorney Docket No. 45240-105719

Exhibit A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

<i>Group:</i>	1639	}
<i>Confirmation No.:</i>	5133	}
<i>Application No.:</i>	10/612,298	}
<i>Invention:</i>	PEPTIDES COMPRISING AROMATIC D-AMINO ACIDS AND METHODS OF USE	}
<i>Applicant:</i>	Byron Anderson	}
<i>Filed:</i>	July 2, 2003	}
<i>Attorney</i>		}
<i>Docket:</i>	45240-105719	}
<i>Examiner:</i>	Christopher M. Gross	}

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Krzysztof Darlak, am not a co-inventor of the patent application captioned above. I am an expert in peptide and peptide library syntheses. I have 25 years of experience in the field of peptide chemistry. When I was employed at Peptides International as Research Director I was the person who made the libraries that Dr. Anderson designed and directed the constructs of, and which he purchased from the company and as used in Dr. Anderson's patent application. I am currently employed as Senior Technical Manager of Chemical Operations at Creosalus, Inc.. My Resume is attached.
2. I have read the patent application captioned above.
3. I understand that the examiner does not believe that Dr. Anderson describes combinatorial libraries of D-peptides in his patent application, where the length of each

D-peptide is from three to seven D-amino acid residues, and where at least 68% of the D-peptides have at least 3 aromatic D-amino acid residues. I have read the patent application and I have read the document "Amendment and Response to Office Action Mailed April 2, 2009," and I understand and agree with the calculations of Dr. Anderson as described on pages 5, 6 and 7 of that latter document.

4. I understand that the examiner doubts that a person familiar with combinatorial libraries, such as me, could only make a pentapeptide library that satisfies the requirements in paragraph 3 in my Declaration, not libraries of different lengths of D-peptides, such as the tri-, tetra-, hexa- and hepta-peptide libraries.
5. With the information in Dr. Anderson's patent application, my knowledge, and in accordance with the information in Lebl et al., "One-Bead-One-Structure Combinatorial Libraries," cited in the patent application, I could make libraries of 3, 4, 5, 6 and 7 amino acids where at least 68% of the D-peptides have at least 3 aromatic D-amino acid residues.
6. The libraries of 3 to 7 amino acid residues would be made as follows all using the split synthesis method as described in Lebo et al.: the tri-peptide library would use the 3 aromatic D-amino acids of D-phenylalanine, D-tyrosine and D-tryptophan; the tetra-peptide library would use the 3 aromatic amino acids and either glycine or D-alanine; the penta-peptide library would use the 3 aromatic amino acids and glycine and D-alanine; likewise the hexa- and hepta-peptide libraries would use the same amino acids as the penta-peptide library. The resulting libraries would all contain at least 68% of all sequences having 3 or more of the aromatic amino acids listed above.
7. I believe that Dr. Anderson has made an important and unique discovery that short peptide sequences containing 3 or more aromatic amino acids can display the properties of high affinities and specificities to many proteins of biologic and medical interest, and that such peptides will find many applications in diagnostic and therapeutic uses. The combinatorial libraries he describes is an efficient experimental approach to identifying such aromatic rich peptide sequences.
8. I hereby declare that all statements made herein are of my own knowledge, and that all statements are made on information believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'K. Darlak', written in a cursive style.

Name: Krzysztof Darlak

Title: Senior Technical Manager

Date: August 28, 2009

Krzysztof Darlak

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Career summary

Versatile, motivating, hands-on technical leader/ organic chemist with a strong emphasis on both solid and solution phase peptide synthesis with extensive experience in Research and Product Development.

Professional Experience

Positions and Employment

2009-present Senior Technical Manager of Chemical Applications, Creosalus, Inc., Louisville, KY
1992-2009 Research Director, Peptides International, Inc., Louisville, KY
1991-1992 Senior Chemist, Peptides International, Inc., Louisville, KY

- Built the research and chemical production department from the ground up at Peptides International Inc., a supplier of high quality building blocks, resins, reagents and custom & catalog peptides.
- Directed research, development and synthesis of the new catalog and custom products.
- Supervised up to five directly reporting chemists, including Ph.D. level chemists.
- Identified new technologies and new classes of compounds via its licensing or collaborations and its development into new products.
- Responsible for designing and outfitting a new research and production facility which was built and became fully operational in 2007.
- Provided customer support for all manufactured products and helped in solving customer's synthetic challenges.
- Direct involvement in all aspects of synthesis, scale up, purification and analysis of manufactured products covering the following but not limited to:
 - Synthesis of unusual building blocks for peptide synthesis (diamino acids, tics, mercaptoacids, pegs etc.)
 - Preparation of the resins for peptide synthesis: functionalization and derivatization of the polystyrene based beads (Merrifield, PAM, MBHA, Wang and others); design, development and scale-up preparation of the beaded polymers via suspension polymerization.
 - Design, development and scale-up of polymer supported reagent Clear-OX, a versatile tool in preparation of disulfide bridged peptides.
 - Solid Phase Peptide Synthesis up via either t-Boc or Fmoc methodologies, solution peptide synthesis or combination of both involving linear, branched and cyclic peptides (lactam, disulfide), phosphonopeptides, lipopeptides, pegylated and biotinylated peptides, Dye-labeled peptides (AMC, pNA, fluorescein, rhodamines, cyanines, Dnp/Amc and others Fret pairs), C-terminally modified

- peptides (aldehydes, alcohols, N-alkyl amides), peptide bond modified peptides ($\Psi[\text{CH}_2\text{S}]$, $\Psi[\text{COO}]$, $\Psi[\text{CH}_2\text{NH}]$), up to 100g scale
- Combinatorial peptide synthesis; preparation of soluble and solid support libraries, utilization of unusual resins.
 - Other skills: mass spectrometry, ran and maintained Applied Biosystems Mariner (ES-MS), HPLC, FT-IR, UV-VIS, polarimeter, large scale HPLC purification systems (NovaSep, up to 110 mm dia. columns), large scale solid phase peptide synthesis (50 L), large scale evaporators (20 L), large scale solution synthesis (100 L reactors).

Academic Appointments

1988-1990 Senior Postdoctoral Research Associate, Dept. of Chemistry, University of Louisville, Louisville, KY

1985-1987 Postdoctoral Research Associate, Dept. of Chemistry, University of Louisville, Louisville, KY

1984-1988 Assistant Professor, Institute of Chemistry, University of Gdansk, Gdansk, Poland

1984-1984 Postdoctoral Research Associate, Institute of Pharm. Chemistry, University of Ferrara, Ferrara, Italy

1981-1984 Research Assistant, Institute of Chemistry, University of Gdansk, Gdansk, Poland

Education

1981 M.S. Organic Chemistry (with honors), University of Gdansk, Gdansk, Poland

1984 Ph.D. Organic Chemistry, Prof. Zbigniew Grzonka University of Gdansk, Gdansk, Poland

Other Experience and Professional Membership

American Peptide Society

European Peptide Society

Patents

A. F. Spatola, K. Darlak, J. J. Wen, P. Romanovskis, "Cyclic peptide mixtures via side chain or backbone attachment and solid phase synthesis," December 28, 1999, United States Patent #6,008,058.

A. F. Spatola, K. Darlak, and R. D. Gray, "Peptide derivatives of collagenase inhibitors," April 1, 1997, United States Patent #5,616,605.

R. D. Gray, A. F. Spatola, K. Darlak, "Peptide derivatives of collagenase inhibitors," February 7, 1995, United States Patent 5,387,610.

Peer-Reviewed Publications

1. K. Darlak, Z. Grzonka, "Synthesis of Dermorphin," *Pol. J. Chem.*, **56**, 1201 (1982).
2. K. Darlak, Z. Grzonka, P. Janicki, A. Czlonkowski, S. W. Gumulka, "Structure-Activity Studies of Dermorphin and its 1-Substituted Analogues," *J. Med. Chem.*, **26**, 1445-1447 (1983).
3. K. Darlak, Z. Grzonka, P. Janicki, A. Czlonkowski, S. W. Gumulka, "Structure-Activity Relationships of Dermorphin," in *Peptides*, 1982, K. Blaha, P. Malon, Eds., W. De Gruyter, Berlin, 1983, pp 502-504.
4. K. Darlak, Z. Grzonka, P. Janicki, S. W. Gumulka, "Structure-Activity Studies of Dermorphin. The Role of Side Chains Amino of Acid Residues on the Biological Activity of Dermorphin," *Peptides*, **5**, 687-689 (1984).
5. Z. Grzonka, F. Kasprzykowski, E. Kojro, K. Darlak, P. Melin, F. Fahrenholtz, P. Crause, R. Boer, "Arginine-Vasopressin Analogues Modified in Positions 1, 2, 4, 7, and 8. Synthesis and Biological Activities," in *Peptides*, 1984, U. Ragnarson, Ed., Wiksell International, Stockholm, 1984, pp 419-422.
6. F. Toma, S. Fermandjian, K. Darlak, Z. Grzonka, "Conformational Studies of Dermorphin," *Spectros. Int. J.*, **3**, 465-472 (1985).
7. F. Toma, V. Dive, S. Fermandjian, K. Darlak, Z. Grzonka, "Preferred Solution and Calculated Conformations of Dermorphin and Analysis of Structure-Conformation-Activity Relationships in Series[Alan]-Dermorphin," *Biopolymers*, **24**, 2417-2430 (1985).
8. I. Gryczynski, A. Kawski, K. Darlak, Z. Grzonka, "Intramolecular Electronic Excitation Energy Transfer in Dermorphin and Its Analogues," *J. Photochem.*, **30**, 371-377 (1985).
9. Z. Grzonka, F. Kasprzykowski, E. Kojro, K. Darlak, P. Melin, F. Fahrenholtz, P. Crause, R. Boer, "Arginine-Vasopressin Analogues with High Antidiuretic-Vasopressor Selectivity. Synthesis, Biological Activity, and Receptor Binding Affinity of Arginine-Vasopressin with Substitutions in Positions 1, 2, 4, 7, and 8." *J. Med. Chem.*, **29**, 96-99 (1986)
10. Krzysztof Darlak, Glen Franklin, Philip Woost, Elaine Sonnenfeld, Daniel Twardzik, Arno Spatola, and Gregory Schultz, "Assessment of Biological Activity of Synthetic Fragments of Transforming Growth Factor- α ," *J. Cell. Biochem.*, **36**, 341-352 (1988).
11. Arno F. Spatola and Krzysztof Darlak, "Amide Bond Surrogates: Pseudopeptides and Macrocycles," *Tetrahedron*, **44**, 821-833 (1988).
12. Krzysztof Darlak, Zbigniew Grzonka, Severo Salvadori, Roberto Tomatis, "Synthesis and Biological Activity of Some Cyclic Dermorphins," *Pol. J. Chem.*, **4-6**, 453-458 (1988).
13. Krzysztof Darlak, Deanne E. Benovitz, Arno F. Spatola, and Zbigniew Grzonka, "Dermorphin Analogs: Resistance to In Vitro Enzymatic Degradation Is Not Always Increased by Additional D-Amino Acid Substitutions," *Biochem. Biophys. Res. Commun.* **156**, 125-130 (1989).
14. Arno F. Spatola, Mohamed K. Anwer, Krzysztof Darlak, Fernando Formaggio, Claudio Mapelli, Douglas B. Sherman, Thomas F. Burks, and William S. Wire, "Cyclic Peptides and Pseudopeptides," *Peptides* 1988 (Gunther Jung and Ernst Bayer,

- eds.) Walter de Gruyter & Co., Berlin, 1989, pp. 646-648. Krzysztof Darlak, Zbigniew Grzonka, Arno F. Spatola, Deanne E. Benovitz, Thomas F. Burks, and William S. Wire, "Synthesis, Biological Activities, and Enzymatic Stabilities of Dermorphin Pseudopeptides," *Peptides* 1988, (A. Aubry, M. Marraud, and B. Vitoux, eds.), John Libbey Eurotext Ltd., 1989, pp. 634-636.
15. Hong Dang, Glen Franklin, Krzysztof Darlak, Arno F. Spatola, and Steven R. Ellis, "Discoordinate Expression of the Yeast Mitochondrial Ribosomal Protein MRP1," *J. Biol. Chem.*, **275**, 7449-7454 (1990).
 16. Krzysztof Darlak, Robert B. Miller, M. Sharon Stack, Arno F. Spatola, and Robert D. Gray, "Inhibition of Pig Synovial Collagenase by Substituted Amide and Peptide Derivatives of the Leucine Analogue, 2-[(R,S)-Mercaptomethyl]-4-methylpentanoic Acid," *J. Biol. Chem.*, **265**, 5199-5205 (1990).
 17. Arno F. Spatola, Krzysztof Darlak, William S. Wire, and Thomas F. Burks, "The Effect of Chirality and Polarity within the Peptide Backbone," *Peptides--Chemistry, Structure, and Biology. Proceedings of the Eleventh American Peptide Symposium* (Jean E. Rivier and Garland R. Marshall, eds.), ESCOM, Leiden, 1990, pp. 334-336.
 18. Krzysztof Darlak, Thomas F. Burks, William F. Wire, and Arno F. Spatola, "Small Ring Enkephalins," *Peptides* 1990 (E. Giralt and D. Andreu, eds., ESCOM, Leiden, 1991, 401-403.
 19. Zbigniew Grzonka, Franciszek Kasprzykowski, Lucyna Lubkowska, Krzysztof Darlak, Theresa A. Hahn, and Arno F. Spatola, "In Vitro Degradation of Some Arginine Vasopressin Analogs by Homogenates of Rat Kidney, Liver, and Serum," *Peptide Research*, **4**, 270-274 (1991).
 20. Robert D. Gray, Krzysztof Darlak, and Arno F. Spatola, "Design and Synthesis of Inhibitors of Porcine Synovial Collagenase and Gelatinase," *Inhib., Proc. Matrix Metalloproteinase Conf.*, 307-308 (1992).
 21. Arno F. Spatola, Krzysztof Darlak, Stefano Pegoraro, Kanchan Nijhawan, Michele Anzolin, Leszek Lankiewicz, and Robert D. Gray, "Peptide Inhibitors of Matrix Metalloproteases," *Proceedings of the Twelfth American Peptide Symposium* (John A. Smith and Jean Rivier, eds.), ESCOM, Leiden, 1992, pp. 820-821.
 22. Deanne E. Benovitz, Krzysztof Darlak, Wieslaw A. Klis, Elias Klein, and Arno F. Spatola, "Membrane Based Peptide Synthesis (MBPS)" *Peptides, Chemistry, Structure and Biology, Proceedings of the Thirteenth American Peptide Symposium*, Robert S. Hodges and John A. Smith, eds., ESCOM, Leiden, 1994, pp. 136-137.
 23. Krzysztof Darlak, Peteris Romanovskis, and Arno F. Spatola, "Cyclic Peptide Libraries," *Peptides, Chemistry, Structure and Biology, Proceedings of the Thirteenth American Peptide Symposium*, Robert S. Hodges and John A. Smith, eds., ESCOM, Leiden, 1994, pp. 981
 24. Seonggu Ro, Qin Zhu, Chang-Woo Lee, Murray Goodman, Krzysztof Darlak, Arno F. Spatola, Nga N. Chung, Peter W. Schiller, Annika B. Malmberg, Tony L. Yakksh, and Thomas F. Burks, "Highly Potent Sidechain-Mainchain Cyclic Dermorphin-Deltorphin Analogs: An Integrated Approach Including Synthesis, Bioassays, NMR Spectroscopy and Molecular Modeling," *J. Peptide Sci.*, **1**, 157-174 (1995).
 25. Carol Feehan, Krzysztof Darlak, J. Kahn, B. Walcheck, Arno F. Spatola, and Takashi Kei Kishimoto, "Shedding of the Lymphocyte L-Selectin Adhesion Molecule Is Inhibited by a Hydroxamic Acid-based Protease Inhibitor," *J. Biol. Chem.*, **271**,

7019-7024 (1996).

26. Arno F. Spatola, Krzysztof Darlak, and Peteris Romanovskis, "Cyclic Peptide Libraries: Reducing Epimerization in Medium Sized Rings During Solid Phase Synthesis," *Tetrahedron Letters*, **37**, 591-594 (1996).
27. Bruce Walcheck, Julius Khan, Joseph M. Fisher, Bruce B. Wang, R. Spencer Fisk, Donald G. Payan, Carol Feehan, Raj Betageri, Krzysztof Darlak, Arno F. Spatola, and Takashi Kei Kishimoto, "Neutrophil rolling altered by inhibition of L-selected shedding in vitro," *Nature*, **380**, 720-723 (1996)
28. Narayan Chatla, Krzysztof Darlak, and Arno F. Spatola, "Applications of CLEAR Resin for Solid Phase Organic Synthesis: Comparison with Polystyrene Based Resin," *Peptides 1996, Proceedings of the European Peptide Symposium, 1997*.
29. K. Darlak, M. Darlak, and A. F. Spatola, "Disulfide oxidation in water: investigation of CLEAR supports for on-resin cyclization," *Peptide Science - Present and Future. Proceedings of the 1st International Peptide Symposium*, (Y. Shimonishi, Ed.) Kluwer Academic Publishers, 1999, pp. 584-586.
30. Arno F. Spatola, Krzysztof Darlak, and Peteris Romanovskis, "Synthesis of peptides with sulfur containing amide bond replacements," in: *Synthesis of Peptides and Peptidomimetics*, Houben-Weyl, Stuttgart, 2003, pp 458-49
31. Krzysztof Darlak, Andrzej Czerwinski, Mirosława Darlak, DeAnna W. Long, Francisco Valenzuela, Arno F. Spatola, and George Barany. CLEAR-OX™: A New Polymer-Supported Reagent for the Preparation of Disulfide-Bridged Peptides. In "Peptide Revolution: Genomics, Proteomics & Therapeutics. Proceedings of the Eighteenth American Peptide Symposium" (Michael Chorev & Tomi K. Sawyer, eds.), American Peptide Society, San Diego, 2004, pp. 59-60.
32. Krzysztof Darlak, Andrzej Czerwinski, Mirosława Darlak, DeAnna W. Long, Francisco Valenzuela, Arno F. Spatola, and George Barany. Multi-Gram Preparation of Disulfide-Bridged Peptides Under Mild Conditions Using CLEAR-OX™. In "Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Libraries, 2004: Collected Papers of the Eighth International Symposium" (R. Epton, ed.), Mayflower Worldwide Ltd., Kingswinford, England, 2004, pp. 213-216.
33. Krzysztof Darlak, DeAnna Wiegand Long, Andrzej Czerwinski, Mirosława Darlak, Francisco Valenzuela, Arno F. Spatola, and George Barany, "Facile Preparation of Disulfide-Bridged Peptides Using the Polymer-Supported Oxidant CLEAR-OX™ " *J. Peptide Research*, **63** , 303-312 (2004).
34. Anne L. Carenbauer, Matthew.R. Cecil, Andrzej Czerwinski, Krzysztof Darlak, Mirosława Darlak, DeAnna W. Long, Francisco Valenzuela, and George Barany, "Microwave-Assisted Solid-Phase Peptide Synthesis (MW-SPPS) on CLEAR Supports." In *Understanding Biology Using Peptides*, (S.E. Blondelle, ed), Proceedings from the 19th American Peptide Symposium, American Peptide Society, pp. 146-147 (2005).
35. B. Witek, E. Ochwanowska, J. Rafay, A. Kolataj, P. Chrenek, K. Suvegova, R. Jurcik, A. Sirotkin, K. Darlak, "Effect of ghrelin on activities of some lysosomal hydrolases in rabbits" *Neuroendocrinology Letters*, **26**, 397-400 (2005).
36. A.V. Sirotkin, A. Benco, A. Tandlmajerova, D. Vasicek, J. Kotwica, K. Darlak, F. Valenzuela, "Transcription factor p53 can regulate proliferation, apoptosis and

- secretory activity of luteinizing porcine ovarian granulosa cell cultured with and without ghrelin and FSH" *Reproduction*, **136**, 611-618 (2008).
37. A.V. Sirotkin, P. Chrenek, K. Darlak, F. Valenzuela, Z. Kuklova "Some Endocrine Traits of Transgenic Rabbits. II. Changes in Hormone Secretion and Response of Isolated Ovarian Tissue to FSH and Ghrelin" *Physiological Research*, **57**, 745-751 (2008).
38. M. Meszarosova, A.V. Sirotkin, R.Grossmann, K. Darlak, F. Valenzuela, "The effect of obestatin on porcine ovarian granulosa cells" *Animal Reproduction Science*, **108**, 196-207 (2008).
39. Matthew R Cecil, Andrzej Czerwinski, Mirosława Darlak,; DeAnna W. Long, Danny McGrew, Timothy L. Morgan, Francisco Valenzuela, George Barany, Krzysztof Darlak, "CLEAR-OX (TM): Synthesis of disulfide-bridged peptides under mild conditions" *Journal Of Peptide Science*, **14**, 67-67 Suppl. S (2008).
40. Alexander V. Sirotkin, Maria Chrenkova, Sona Nitrayova, Peter Patras, Krzysztof Darlak, Francisco Valenzuela, Leonor Pinilla, Manuel Tena-Sempere, "Effects of chronic food restriction and treatments with leptin or ghrelin on different reproductive parameters of male rats" *Peptides*, **29**, 1362-1368 (2008).